REMARKS

The Applicants thank the Examiner in advance for review and reconsideration of this Amendment and accompanying changes. Paragraphs are numbered to correspond to Examiner's Office Action of March 3, 2005 for ease in identification.

Upon entry of this amendment, claims 8-18 are pending and stand rejected, with claims 1-7 and 19-24 having been cancelled. Claims 8-18 are amended herein. Support for these amendments is found throughout the specification and claims as filed, and particularly in the final full paragraph of claim 2, the first full paragraph of page 3, and the table on page 5.

9. Sequence identifiers have been provided in accordance with 37 CFR § 1.82(a). They correspond to the sequence identification listing submitted on September 16, 2002.

10. In accordance with 37 CFR § 1.52(b) (4), the abstract is presented on a separate sheet in this transmission. Entry of this abstract is respectfully requested.

Rejections Under 35 U.S.C. § 112

11-13. Claims 8-13 and 15-24 are rejected for alleged failure to comply with the written description and enablement requirements. It is believed that the currently amended claims will overcome the Examiner's objection and put the pending claims in condition for allowance. The rejections are moot with respect to the cancelled claims. The proposed amendments provide a reasonable limitation that are supported by the specification and are enable without undue experimentation. Furthermore, it could be reasonably expected by one skilled in the art that glycosylated fragments or repeats as claimed in the amended claims would retain the increased binding affinity as set forth in the specification.

It is believed that with the benefit of the specification as amended herein one skilled in the art would be able to make and use a glycosylated peptide as described without

undue experimentation. The Examiner is respectfully asked to consider the following comments on the prior art:

The Examiner asserts that the function of an otherwise undefined peptide comprising the amino acids of SEQ. ID NO: 3 cannot be reliably and accurately predicted. That the exact folding of a polypeptide chain into its ultimate protein structure cannot reliably be predicted by the amino acid sequence alone is referenced by Skolnick et al. (*Trends in Biotechnology* 2000; **18**: 34-39) and Bowie et al. (*Science* 1990; **257**: 1306-1310). The Applicants respectfully refer to the named articles to describe the general problem of comparing primary structure and protein function in an era where the genomes of a number of species are already completely sequenced. It is agreed that this is the case for amino acid sequences which fold into tertiary and/or quaternary structures, providing a vast number of nonlinear epitopes on the protein surface. This is due to the fact that formerly-separated parts of the primary structure are suddenly adjacent after the folding process in a stabilized three dimensional structure, revealing a complex structural landscape on the surface that can be bound by different types of antibodies.

The ultimate folding of an amino acid sequence is dependent on its sequence length. Short amino acid sequences or peptides are capable of forming structures on their own, usually alpha helixes or beta sheets. The shortest known peptides that can fold into a beta sheet protein structure, WW-domains, still have to be more than 30 amino acids in length as prerequisite for the folding process. Illsley et al. *(Cell Signal* 2002; 14: 183-189, cited by the Applicants). In addition, other natural peptides form tertiary peptide structures. For example, the leucine zipper of eukaryotic bZip transcription factors, PDZ or SH3 domains do not usually fold if the sequence length is shorter than 30 amino acid residues Bal et al.*(Angew. Chem Int. Ed. Engl* 2005; **44:** 2852-2869 cited by the Applicants); Mullan (*Brief, Bio and Form* 2004; **5**: 71-74

cited by the Applicants). Therefore, it is highly unlikely that the presently disclosed SEQ. ID NO: 3 can fold singly to a tertiary structure, and therefore would not be able to form an indefinite number of epitopes on its surface.

Short peptides can form structure by their interaction with proteins, in particular by folding into the recognition element of an antibody. The structural properties of the peptide epitope can only be achieved when in contact with a stabilizing antibody surface, since short peptides usually do not form a structure on their own in solution. It can be reasonably assumed that the disclosed sequence, SEQ. ID NO: 3, can only form linear epitopes due to its very short sequence. Furthermore, SEQ. ID NO: 3 includes proline, an amino acid providing a tertiary amide in the backbone which normally disturbs the formation of sheets and/or helical secondary structure.

The antibody recognition of a linear peptide, usually 6 to 12 amino acids in length, is mainly driven by electrostatic or hydrophobic interactions of key residues in the linear epitope interacting with the side chains of the antibody binding pocket. The peptide sequence is very specifically recognized by the antibody. Because the mutation of only one of the key residues is normally sufficient for the peptide not to be realized by the antibody it is known, in the alternative, that a goal oriented mutation of a peptide can result in a synthetic epitope providing a far higher affinity to bind to the antibody than the natural epitope. Because of these foregoing reasons, it was totally surprising to the Applicants that a glycosylation of the threonine reside inside the sequence, SEQ. ID NO: 3, results in an epitope having a higher affinity towards the investigated anti MUC1 antibody. Because glycosylations of amino acids are normally post translational modifications, they are usually not predicted by sequence. In particular, the glycosylation of the respective threonine reside in the immunogenic epitope of MUC1 was not

known previously, and therefore the results were totally not at all expected. Because of these foregoing reasons, the Applicants respectfully request that the rejection of claim 1 be overcome by the currently amended claim supplied herein.

The Examiner states several different articles discussing controversy surrounding the immune response against tumor antigen and its benefits for the patients. The Applicants respectfully believe that the cited publications cast a positive light on the possibilities of tumor vaccinations. For instance, Yamshchikov et al. (*Clinical Cancer Research* 2001; **7**: 909-916) teaches that a patient having a peptide isolated and subsequently being vaccinated by that peptide has remained disease free for six years (page 909, first column). The Applicants do not challenge the fact that certain antigens could be only partially recognized in a patient due to the different HLA types of major histocompatibility complexes. As Yamshchikov, et al., reports, it is a desirable characteristic found in a survivor that may serve as the reasonable goal for future clinical trials of melanoma vaccines or other immunotherapy (page 915, second column).

Bocchia, et al. (*Hematologica* 2000; **85**: 1172-1206), reports that controversial findings of the T-cell response may be due to techniques used to monitor the state of immunization of cancer patients and emphasizes that there can be an opportunity to vaccinate the susceptible subject against their possibly recurring cancer in the near future (page 1172, second column). Bodey et al. (*Anti Cancer Research* 2000; **20**: 2665-2676), casts a critical light on tumor vaccination due to the possibility of faulty antigen presentation (page 2665, first column). Also, Gura (*Science* 1997; **278**: 1041-1042) provides a critical comment on the future of drug discovery for cancer and is cited by the Examiner.

However, these criticisms have been largely overcome since the latest developments in literature, immunological and clinical results of the latest trials in tumor vaccination have seen

overwhelmingly positive results. Mosolas et al. (*Ann. Oncol.* 2005; **16**: 847-862 cited by the Applicants), for example, reviews the therapeutic vaccination trials in patients with gastrointestinal malignancies over the last five years. More than 2000 patients have been vaccinated with tumor antigens (self antigens). The procedure is safe and no autoimmune disorders have been observed after more than four years follow-up in a substantial number of patients. Humoral and cellular tumor antigens specific immune responses were induced. The correlation between immune responses and prolonged overall survival was seen in several studies (page 847, abstract).

Ezzel was critical of tumor vaccination ten years ago, (*Journal of NIH Research* 1995; **7**: 46-49) but still ended his article by noting, "[T]he current developments in cancer immunology cannot even be imagined five years ago. What comes next is anybody's guess." A similar statement is also given by Spitler in the same year (*Cancer Biotherapy* 1995; **10**: 1-3). "Now that the active components of the vaccines have been identified and purified, we are approaching the stage in technology where the interferons were at the beginning of the 1980s. The decade of the vaccines may finally have arrived!" (page 7, second column).

And in fact, the recent published clinical results underline the beneficial progress being made in the last ten years as summarized, for example, by Kaplan (*Drugs Today* 2004 Nov.; **40** (11): 913-929, cited by the Applicants). "Recent progress in immunology and tumor biology has allowed for the development of new vaccine strategies and approaches to enhance clinical efficiency" (page 913, abstract). As Boon (*Advances in Cancer Research* 1992; **58**: 177-210) notes, "[T]he identification of tumor rejection antigens will undoubtedly provide the possibilities such as immunizing with cells, that over expresses the antigen, and immunizing with purified,

peptide antigen presenting cells, such as dendritic cells that have been pulsed with the peptide." (page 206, first paragraph).

More recently the same author has published results in tumor regression using these antigens (Lonchay et al. 2004 Proc. Natl. Acad. Sci. USA **101**: 14631-14638, cited by the Applicants). The article emphasizes "it is possible that even those CTL (cyto-toxic lymphocyte) responses that are below our present protection level can trigger a sequence of events that leads to tumor regression" (abstract). Lee et al. (*Journal of Immunology* 1999; **163**: 6292-6300) vaccinated using the peptide GP 100: 209-217, and it was later shown that the poor immunogenicity of the antigen "is due to the instability of the peptide MHC complex rather than to the continual deletion of the polarization of self reactive T cells" (abstract Yu et al. 2004 J. Clin. Invest. **114**: 551-559, cited by the Applicants). Interestingly Yu, et al. report in this article that a mutant of this peptide is significantly more immunogenic that the native peptide sequence. Furthermore, the antigen P 369-377 used by Zaks, et al. (*Journal of Immunology Therapy*; 2000; **23**: 643-653) was later found to be useful as a tumor vaccine when it was combined with poly(lactide-co-glycolide) microspheres. Mossmann, et al. (*Vaccine* 2000; **23**: 3543-3554, cited by the Applicants).

The use of IL-12 for tumor vaccination that is described by Gao et al., (*Journal of Immunotherapy* 2000; **23**: 643-653) that was shown to be effective in the CSA1M, but not in the METH A CSA1M-variant tumor models, has recently been used successfully for tumor suppression if combined with H-2K restricted peptide (Shimizu et al. 2004 *Journal of Immunotherapy*; **27**: 265-272, cited by the Applicants). Also the results of Lu et al. (*Cancer Research*; **62**: 5807-5812, 2002) using insilico predicted peptides derived from PMSA have been

successful in respect to their ability to stimulate T lymphocytes Kobayshi et al. (*Clin. Cancer Res.* 2000; **9**: 5386-5393, cited by the Applicants).

Just recently it has been shown that peptides derived from a tumor associated antigen (TAA) of MUC1 can induce immunologic responses in patients with advanced metastatic breast and ovarian cancers, Wierecky et al. (*Cancer Immunol. Immunother.* Apr. 28, 2005 [E. Pub. ahead of print], cited by the Applicants). Furthermore, recently the advantageous characteristics of glycosylated peptides derived from MUC1 have also been confirmed, Karsten et al. (*Glycobiology* 2004; **14**: 681-692, cited by the Applicants); Xu et al. (*J. Exp. Med.* 2004; **199**: 707-716, cited by the Applicants).

Because no phase 1-3 treatments of patients have been disclosed in the description, the Applicants cancel claims 19-24 but respectfully ask the Examiner to take the inventive benefit into account and to grant a patent for the currently amended claims.

Rejections Under 35 U.S.C. § 102

14-16. The applicant respectfully traverses the Examiner's 35 U.S.C. § 102 rejections as applied to the pending claims in light of the attached translation of the specification of German priority application DE 197 58 400.4 filed on December 30, 1997. Also attached is a Certificate signed by Dr. Friedrich Baumbach attesting to the accuracy of the translation of the German document. This application predates the Karsten publication cited by the Examiner and establishes a foreign priority date of December 30, 1997, for the instant application; the earlier priority date disqualifies Karsten as a reference. The rejection should be withdrawn and the claims allowed.

CONCLUSION

It is respectfully believed that the present application is in condition for examination and allowance. Early notice to this effect is earnestly solicited. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this Application, the Examiner is invited to telephone the undersigned at the number provided.

Please note that the correspondence address for this application is now the address for Customer Number 23464.

Respectfully submitted,

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Tumor vaccines for MUC1-positive carcinomas

Field of invention

The invention relates to tumor vaccines of a new type, based on the molecular structure of human epithelial mucin (MUC1). The invention can be used for the immunotherapy of carcinomas.

Background

Epithelial mucins are glycoproteins with repetitive amino acid sequences and a high proportion of carbohydrates which are partially bound to membranes, partially secreted and are to be found on many glandular epithelia. The epithelial mucin known best is the membrane-bound MUC1, described also as PEM, PUM, EMA, MAM-6, PAS-0 or episialine (Finn, O. et al., Immunol. Reviews 145:61, 1995) the extracellular part of which consists of a variable number of repeating units of 20 amino acids, the so-called tandem repeats. The MUC1 is not a tumor specific molecule *per se*; its suitability as tumor antigen is based on the fact that its carbohydrate portion is qualitatively and quantitatively changed in tumors (Burchell, J. and Taylor-Papadimitriou, J., Epith. Cell Biol. 2:155, 1993). Here, new epitopes appear which are detected by the immune system (humoral and cellular defense).

After operatively removing the primary tumor (or after a radiation or chemotherapy) one, as a rule, has to proceed on the assumption that tumor cells still remain in the body (minimal residual disease). These tumor cells which represent a potential danger, are combated by various endogenic mechanisms the efficiency of which may be intensified by an adjuvant immunotherapy. The most effective adjuvant immunotherapy is vaccination. Here, two prerequisites are present: first, a suitable target antigen (epitope) has to be present on the tumor cells, and second that it should be possible to prepare a form of vaccine that is immunogenically as strong as possible, most suitably in a synthetic form.

Non-glycosylated oligo-repeat peptides of MUC1 represent a suitable target antigen in a number of frequently occurring carcinomas (Apostolopoulos, V. and McKenzie, I.F.C., Crit. Rev. Immunol. 14:293, 1994). The immunodominant region of MUC1 is the PDTRPAP motif which occurs on each tandem repeat. However, experiments carried out so far to develop a vaccine on the basis of an individual tandem repeat have not been successful. According to the present state of knowledge a minimum length of the peptide which will be reached only in 3-5 tandem repeats is required for achieving the immunogenic conformation of the peptide (Fontenot, J.D. et al., J. Biomol. Struct. Dyn. 13:245, 1995).

Brief description of the drawing

The invention is described with reference to the drawing wherein

Fig. 1 shows bending of the anti-MUC1-antibody A76-A/C7 on the glycopeptides A1-A9 and A11-A12; and

Fig. 2 shows bending of the anti-MUC1-antibody MFO6 on the glycopeptides A9 and A11-A12.

Description of the invention

It is an object of the present invention to develop tumor vaccines on the basis of the molecular structure of human epithelial mucin MUC1 for combating tumor cells which remain in the body after other therapies.

In the immunological investigation of synthetic glycopeptides which correspond to a tandem repeat of the MUC1 there it was surprisingly detected that the glycosylation of threonine in the immunodominant PDTRPAP region with α-GalNAc significantly increases the antigenicity. So far we proceeded on the theory that this position is not glycosylated in native MUC1, because it was assumed previously that, as a rule, glycosylation hindered the

identification of peptide epitopes and the results of *in vitro* glycosylation experiments (Stadie, T. et al., Eur. J. Biochem. 229: 140 (1995). Latest investigations (Mueller, S., et al., J. Biol. Chem. 272:24780, 1997), however, showed that threonine may be well glycosylated *in vivo* in the PDTRPAP variant. From these latest results the conclusion was drawn that the antigenicity (and in this connection also the immunogenicity) of the MUC1 tandem repeat will be significantly increased by glycosylating threonine in the PDTRPAP variant by GalNAc or by a short oligosaccharide. Thus, the immunogenic conformation of the immunodominant region is already reached by an individual tandem repeat. The antigenicity of the glycosylated PDTRPAP variant in a monorepeat exceeds even that of the oligomeric non-glycosylated peptide.

This discovery develops tumor vaccine mostly but not exclusively from human epithelial mucin MUC1 various molecular sizes glycosylated on threonine of the PDTRPAP variant by GalNAc, or a short oligosaccharide. That objective is met by synthetic peptides of various lengths, suitably a synthetic peptide having a length of at least 20 amino acids, and modified by human epithelial MUC1 glycosylated threonine and containing the immunodominant PDTRPAP region. The glycolyzation can be suitably carried out by a monosaccharide, acetylgalactosamine (GalNAc), a short-chained oligosaccharide, and the disaccharide GalB-1, 3GalNAc.

The tumor vaccine of the present invention can be suitably administered to a patient against mammary, colorectal or pancreatic carcinomas.

The invention is explained in greater detail by reference to the following example.

Example

Antigenicity of synthetic, MUC1-derived glycopeptides

In the following experiment, the binding is investigated of monoclonal antibodies against the immunodominant PDTRPAP variant of the epithelial mucin to synthetic glycopeptides of this mucin in a solid-phase immunoassay (ELISA). The glycopeptides marked as A1 to A12 are indicated in the following Table. They correspond to an overlapping tandem repeat of MUC1 and contain 5 potential glycosylating sites (3 x threonine, 2 x serine); A1-A9 contain an additional alanine. The glycopeptides differ by the number and position of the glycosylating sites as specified in the Table. A1-A9 carry the Thomsen-Friedenreich (TF) antigen as glycan ß-D-Gal(1-3)α-D-GalNAc-O-R whereas A11 and A12 carry only α-GalNAc-O-R (the Tn antigen). The antibodies used are: **A76-A/C7** (mouse, IgG1, epitope: APDTRPAP) and **MFO6** (mouse, IgG1, epitope DTRPAP) (see: Rye, P.D., Price, M.R., eds., ISOBM TD-4 International Workshop on Monoclonal Antibodies against MUC1, Tumor Biol. 19, Suppl. 1, 1998).

Table: Synthetic glycopeptides; the peptide corresponds to the basic structure of the epithelial mucin (MUC1). The immunodominant region is underlined, as also shown in the drawing.

A: Glycosylation with TF:

A—H—G – V—T—S—A—P—D—T—R—P—A—P—G—S—T—A—P—P—A

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

Peptide # glycosylated in position:

A1	5
A2	10
A3	17
A4	6
A5	16
A6	5, 17
A7	5, 16, 17
A8	5, 6, 16,17
A9	5, 6, 10, 16, 17

B: Glycosylation with Tn:

H –G—V—T—S—A—P—D—T—R—P—A—P—G—S—T—A—P—P—A

2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

Peptide # glycosylated in position:

A11	5,17
A12	5, 6, 10, 16, 17

The results show that the peptides glycosylated in position 10 with the two antibodies shown in the example bind significantly stronger than peptides not glycosylated in this position. Glycosylations in other positions have no influence. Substitution by Tn or TF is equal. The binding behavior demonstrated in this example is also shown by other MUC1 antibodies; yet, there are also exceptions. The increased antigenicity of the peptides glycosylated in position 10 can also be shown in inhibition experiments.

The results show that a glycosylation of the immunodominant region of the MUC1 peptide by means of Tn or TF significantly increases the antigenicity.

Certificate

I, Patent Attorney Dipl.-Chem. Dr. Friedrich Baumbach

declare I am competent in the German and English languages and I do hereby certify, that the annexed document is to the best of my knowledge and belief a true and correct translation of

DE 197 58 400.4.

Declared at Berlin
this 6 day of July, 2005.

F. Baumbach

Patent Attorney Dr. F. Baumbach